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Case Report

A case of *Bacillus subtilis* var. *natto* bacteremia caused by ingestion of natto during COVID-19 treatment in a maintenance hemodialysis patient with multiple myeloma

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ABSTRACT

A 70-year-old woman, who started on hemodialysis 7 months before for end-stage renal disease due to diabetic nephropathy and was diagnosed with symptomatic multiple myeloma 1 month before, was admitted to our hospital with critical coronavirus disease 2019 and treated with long-term immunosuppressive therapy such as steroids and tocilizumab. During treatment, *Bacillus subtilis* was detected in the blood cultures. We could not exclude the association of natto (fermented soybeans) with *B. subtilis* var. *natto*, which the patient had been eating every day from 8 days after admission. She was prohibited from eating natto and treated with vancomycin. Later, *B. subtilis* detected in the blood culture was identified as *B. subtilis* var. *natto*, which was identical with those contained in the natto that the patient consumed daily using a next-generation sequencer. Gut dysbiosis due to old age, malignant tumor, diabetes mellitus, end-stage renal disease, and intestinal inflammation caused by severe acute respiratory syndrome coronavirus 2 increased intestinal permeability and the risk of bacterial translocation, causing *B. subtilis* var. *natto* bacteremia. Therefore, careful consideration might be given to the intake of fermented foods containing live bacteria in patients with severe immunocompromised conditions.

1. Introduction

Since the first case of coronavirus disease 2019 (COVID-19) was reported in December 2019, the pandemic continues with no prospect of termination. Oran et al. reported that 33% of people with new coronavirus infections remain asymptomatic, while 81% of patients who develop symptoms have mild infections, 14% have severe infections, and 5% have critical infections [1]. It has been reported that patients with a high risk of severe COVID-19 include those with diabetes mellitus, chronic obstructive pulmonary disease, malignant diseases, chronic kidney disease, post-transplant state, obesity, smoking, and elderly age [1]. Among patients with malignant diseases, patients with hematological malignancy are at a particularly high risk of severe disease [2]. When these high-risk patients become severely ill with COVID-19, they can be treated with strong immunosuppressive therapy, such as steroids and tocilizumab, in addition to antiviral drugs, which further induces an immunocompromised state.

Natto (Fig. 1), which is one of the most common fermented foods in Japan, is made by inoculating steamed soybeans with *B. subtilis* var. *natto* and fermenting it. This microorganism is thought to have beneficial effects on host health when properly ingested, and, because of its low pathogenicity, is rarely the causative agent of infectious disease in humans with normal immunity.

We report a first case of *B. subtilis* var. *natto* bacteremia in an elderly

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Fig. 1. Natto is a typical Japanese fermented food made by inoculating steamed soybeans with *Bacillus subtilis* var. *natto*. The string-like texture of natto is unique to Japan.

patient on hemodialysis with diabetes mellitus and multiple myeloma who developed COVID-19 and, during immunosuppressive therapy, ingested natto daily from 8 days after admission.

2. Case report

A 70-year-old woman started on hemodialysis 7 months before admission for end-stage renal disease due to diabetic nephropathy. One month before admission, she was diagnosed with a pubic bone fracture due to severe pain at the fracture site. A serum protein electrophoresis demonstrated a monoclonal IgG kappa protein of 3150 mg/dL. A bone marrow biopsy showed 38% involvement by abnormal appearing plasma cells, confirmed by CD138 immunohistochemical stain, leading to the diagnosis of symptomatic multiple myeloma. The patient was under consideration for treatment.

The woman, not yet vaccinated against COVID-19, complained of fever, dyspnea, and general malaise, and was hospitalized in our institution. She was diagnosed as COVID-19 by antigen quantification test (Lumipulse® SARS-CoV-2 Ag) and CT scan, revealing ground-glass opacity in both lung fields. Blood tests showed mild inflammation, with a white blood cell count of 4800/μL and a C-reactive protein level of 0.40 mg/dL. On the day of admission, the patient required 2–3 L/min of oxygen. Therefore, favipiravir (3600 mg/day on the first day, 1600 mg/day on the second and subsequent days), dexamethasone (DEX) (6 mg/day), and heparin calcium (5000 units twice daily subcutaneously) were initiated. Due to deterioration on day 4, DEX was increased to 20mg/day and antimicrobials (Tazobactam/Piperacillin) were administered for secondary bacterial pneumonia. Following the improvement, DEX was reduced to 6mg/day, but again, oxygenation generally worsened. From the 14th day, the patient underwent high-flow nasal cannula (HFNC) oxygen therapy and was administered tocilizumab (400 mg/day) and steroid half-pulse therapy (methylprednisolone 500 mg/day for 3 days) intravenously, followed by 10 mg of DEX, which was carefully tapered off by 2 mg every 5 days. On the 20th day, HFNC was eventually completed, and antimicrobial therapy was discontinued. Although the course of COVID-19 was favorable, two sets of blood cultures were performed on the 27th day because of prolonged low-grade fever. Gram-positive rods were detected in one of them and identified as *B. subtilis* by mass spectrometry (Fig. 2a and b). Considering the possibility of contamination, we repeated two sets of blood cultures on the 29th day and confirmed the presence of *B. subtilis* in both blood cultures. Antimicrobial therapy with vancomycin was initiated on the 32nd day. Although the imaging examination did not reveal the invasion site of *B. subtilis*, we could not exclude the association with natto, which the patient had been eating every day from 8 days after admission, from frequent diarrhea after admission. Vancomycin was completed after 14 days of treatment. As the clinical course was favorable, she was transferred to another hospital on the 89th day for rehabilitation.

To investigate whether the bacteria detected in the patient's blood culture were consistent with those contained in the natto that the patient consumed daily, we purchased the same brand of natto and applied some of it to culture on a blood agar medium (Nippon Becton Dickinson, Tokyo, Japan). We analyzed the draft whole-genome of each *B. subtilis* strain detected in the patient's blood and natto using a next-generation sequencer, MiSeq (Illumina). Sequencing depth (coverage) of quality and adapter trimmed reads using Trimmomatic tool (version 0.39) [3] to *de novo* assembled total genome size using SPAdes (version 3.14.1) [4] were 51.2 × (strain TUM20336 isolated from blood) and 51.2 × (strain TUM20337 isolated from the natto). Both strains possessed *B. subtilis* var. *natto* specific genetic elements, IS*4Bsu1* and IS*256Bsu1*, that were analyzed by nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). According to a previous report, core-genome based genetic relationship analysis was performed by reads mapping strategy [5]. Briefly, we trimmed reads mapping to the reference genome, *B. subtilis* var. *natto* BEST195 (accession no. AP011541.2), using bwa version 0.7.17 (-sw option); subsequently, we excluded the estimated homologous recombination genomic region by ClonalFrameML version 1.11 and performed mutation detection by Samtools version 1.9 (mpileup option). No point mutations between TUM20336 and TUM20337 were detected on the core-genome region comparing with the reference genome and 97.6% homology (4,005,283 bp/ 4,105,380 bp) was observed in the core-genome. These results demonstrated that the strains of *B. subtilis*

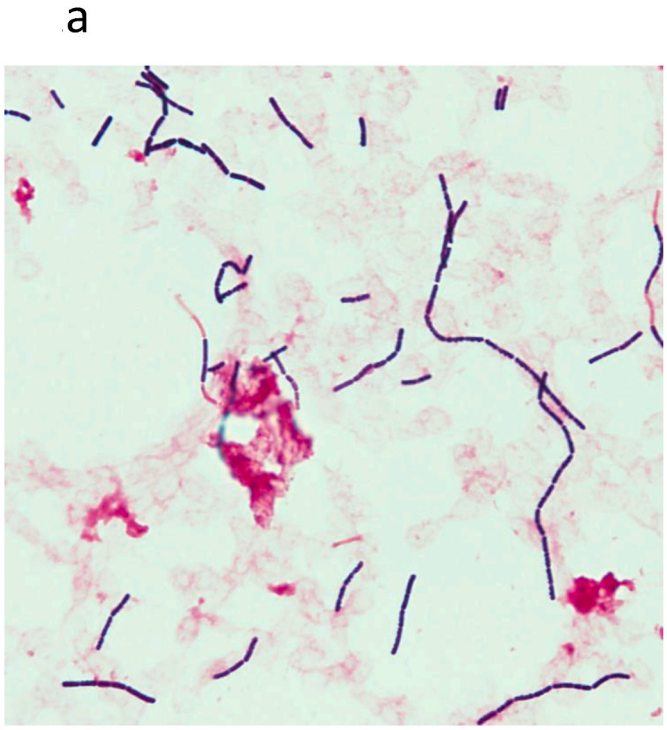

Fig. 2a. Gram staining from the positive blood cultures revealed the presence of Gram-positive rods. *Bacillus subtilis* was isolated from the blood of the patient. Thereafter, *B. subtilis* was identified as *B. subtilis* var. *natto*.

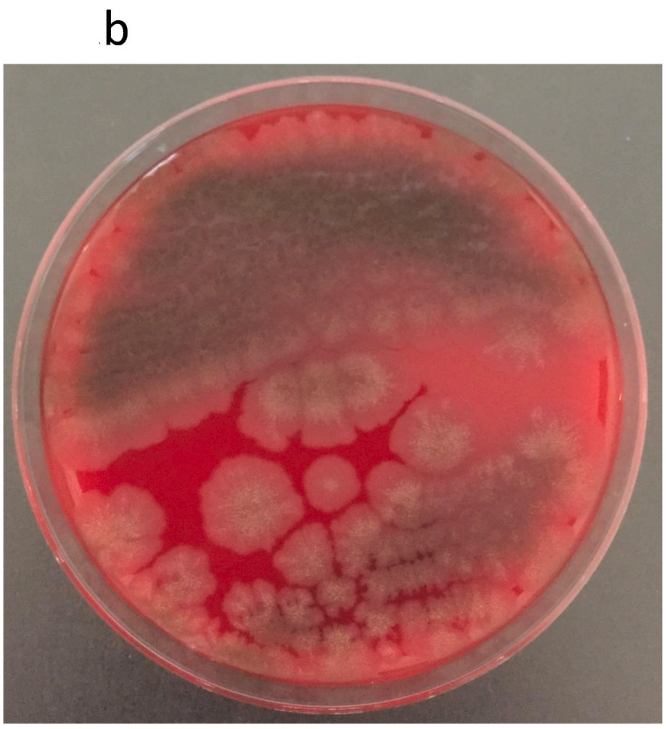

Fig. 2b. Appearances and characteristics of subculture colonies on agar plates of blood after 24 hours incubation of the isolated organism from blood culture.

var. *natto* detected in the blood and natto consumed by the patient were identical.

3. Discussion

We report a case of *B. subtilis* var. *natto* bacteremia resulting from the ingestion of natto in an elderly maintenance hemodialysis patient with diabetes mellitus and multiple myeloma, who developed COVID-19 and received immunosuppressive treatment. It has been reported that the factors relating to COVID-19 severity include diabetes, chronic obstructive pulmonary disease, cancer, hematological malignancy, chronic kidney disease, post-transplantation, obesity, smoking, and age [1,2]. This patient was at high risk of developing severe COVID-19 because of her age, diabetes mellitus, hematological malignancy, and chronic kidney disease, all of which are known as risk factors for severe COVID-19. The patient was in a critical condition and had to be treated with strong immunosuppressive therapy, including long-term steroid treatment and tocilizumab treatment, resulting in a prolonged immunocompromised state. *B. subtilis* is a Gram-positive, aerobic, spore-forming soil bacterium ubiquitous in the environment. It generally has low pathogenicity and is a common cause of contamination even when detected in clinical specimens [6]. On the other hand, septicemia, meningitis, pneumonia, endocarditis, wound infection, and intraocular inflammation caused by *B. subtilis* have been reported [7], mainly in patients with underlying diseases or in an immunocompromised state. Risk factors for the pathogenesis and severity of *B. subtilis* bacteremia include neutropenia, hematologic malignancies, recurrent cancer, the induction phase of chemotherapy, receiving systemic corticosteroids or third generation cephalosporins, and recent hospitalization [7,8]. When *B. subtilis* are isolated from blood cultures in patients with these risk factors, the possibility of true bacteremia should be considered rather than contamination.

B. subtilis var. *natto* is a subspecies of *B. subtilis* and is used to produce natto. Natto is a traditional Japanese fermented food made by inoculating steamed soybeans with *B. subtilis* var. *natto* and fermenting them. The string-like texture of natto is unique to Japan and is characterized by the fact that it is fermented without salt and can be eaten raw. Studies have shown that natto has not only a high nutritional value, but also various functional effects, one of which is the inhibition of the growth of *Clostridium* species, a representative of the so-called "bad" bacteria in the intestines [9]. Daily consumption of natto is expected to maintain the inhibitory effect on the bad bacteria, prevent changes in the intestinal microflora, and improve bowel movements and intestinal regulation.

In addition to natto, the bacteria contained in many fermented foods, which are consumed to have beneficial effects on the body, are known as probiotics. These bacteria are considered to be of low virulence and do not cause infections in humans with normal immunity. However, there are some cases of bacteremia caused by consumption of fermented foods such as yogurt, cheese, and probiotics [10,11]. Many of these cases involved patients with underlying diseases such as malignancy, diabetes mellitus, chronic kidney disease, immunodeficiency, and old age [12]. These diseases are reported to cause alterations in the intestinal microbiota composition and the intestinal environment, the so-called gut dysbiosis. It has been pointed out that gut dysbiosis might impair the intestinal epithelial barrier's structure and function, allowing live bacteria in food to invade the circulatory system by bacterial translocation [13]. Although no reports of bacteremia due to the ingestion of natto have been published, Oggioni et al. reported that administration of probiotic strains of *B. subtilis* in an immunocompromised patient caused *Bacillus* bacteremia [6]. The authors concluded that live bacteria should be used with extreme caution in immunocompromised patients, referring to the long-term persistence of live microorganisms in the intestinal tract.

Moreover, an association between COVID-19 and the gut environment has also been documented. Zerbato et al. reported that intestinal inflammation caused by SARS-CoV-2 causes "leaky gut" syndrome in COVID-19 patients, potentially facilitating the invasion of other bacteria and viruses from the intestine, which may lead to secondary infection [14]. In the context of the present report, it could be concluded that the patient was exposed to an intestinal environment predisposed to changes in the intestinal microbiota and disruption of the intestinal barrier due to multiple factors, including old age, hematological malignancy, diabetes mellitus, end-stage renal disease, COVID-19, and immunosuppressive therapy, developing *B. subtilis* var. *natto* bacteremia due to bacterial translocation. *B. subtilis* var. *natto* can reach the intestine without losing its spore germination ability even when exposed to gastric acid, and remains in the intestinal tract for a long time after ingestion. *B. subtilis* var. *natto* could have been selected based on the microbial substitution by broad-spectrum antimicrobial agents and patient's daily consumption of natto. When the immune system is severely compromised, the risk of consuming of fermented foods containing live bacteria might be carefully evaluated as well as patients after hematopoietic stem cell transplantation are recommended to refrain from eating natto and yogurt [15].

4. Conclusion

B. subtilis var. *natto*, normally acting as an intestinal protector, can cause bacteremia through bacterial translocation due to gut dysbiosis as a result of old age, diabetes mellitus, end-stage renal disease, hematological malignancies, leaky gut caused by SARS-CoV-2, and long-term immunosuppressive treatment for COVID-19. The risk of consuming foods containing live bacteria, such as fermented foods, might be carefully evaluated in such patients.

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Authors' contributions

AY and MR designed for this study. AK and AY drafted the paper. AK, AY, DT, MK, TK, and MR examined the patients, and participated in the decision of the treatment. KK, KA, KY and YI analyzed the samples. All authors critically read and approved the final manuscript.

Informed consent

Informed consent was obtained from the patient for publication of this case report and any accompanying images.

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